AMPHOTERIC BEHAVIOR OF COMPLEX SYSTEMS.

III. The Conductivity of Sulfanilic Acid-Lysin Mixtures.*

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The hypothesis that a mixture of two ampholytes behaves, within certain limits, as an individual (1) with characteristics distinct from either component can also be semiquantitatively tested by certain conductivity experiments. These are based on the observation that the addition of such a substance as gelatin markedly decreases the observed conductivity of a phosphate solution. The magnitude of the decrease is a function of the pH and passes through a minimum at the isoelectric point of the gelatin. In other words, the specific contribution of the gelatin to the measured conductivity is negative. Consider the case of two such ampholytes, A and B. Their separate effects on the conductivities of buffer solutions will be as above, and will be a minimum at their respective isoelectric points. When mixtures of the two are observed, however, their effect is altered due to the fact that through a certain pH range, namely between their respective isoelectric points, there will also be a tendency for mutual "binding" of A and B with a resulting "release" of buffer, resulting in a measured conductivity greater than would be calculated from a knowledge of their behavior when observed separately. This difference between the observed and calculated value of the conductivity may be expected to pass through a maximum at or near what has been termed the isoelectric point of the system.

In case the substances A and B have themselves conductivities comparable with those of the buffer, there is another possibility, which is much more probable, but which cannot be certainly predicted.

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Their specific contributions to the conductivity, when observed separately, may be positive or negative, or may even change sign at some definite pH. One might expect, however, that, if there be any considerable mutual binding tendency between two fairly conducting substances, the measured conductivity will be less at the isoelectric point of the system than would be expected from their separate behaviors, and that this difference would be a maximum at this pH.

To test this idea the conductivities of sulfanilic acid and of lysin were measured in phosphate buffers. The buffers were in all cases 0.02 molal in phosphate. The concentration of the sulfanilic acid and of the lysin were, throughout, the same, the former being 0.020 molal and the latter about 0.023 molal. The mixtures were 0.020 molal in sulfanilic acid and 0.023 molal in lysin. The water employed in the work had a specific conductivity of about 2×10^{-6} reciprocal ohms at room temperature.

The measured conductivities at 25°C., in reciprocal ohms, are given in Table I. The pH values were measured by means of the apparatus used in the titration work described in the preceding paper (1).

From these values pH-conductivity curves were plotted and the conductivities at comparable pH values were read off. These latter values are given in Table II. Obviously the same buffer mixture will not bring sulfanilic acid to the same pH that it will lysin when the buffer concentration is at all comparable to that of the sulfanilic acid or the lysin. It will be seen, however, that for any pH, with constant total phosphate concentration, the concentrations, and thus the conductivities, of the various anion species of the buffer will be the same in all cases unless certain of them tend to be bound by the sulfanilic acid or the lysin. There will, however, be a difference in the sodium ion concentration, and some correction must be made for this. In Table II there is therefore included the total sodium concentration. The correction is made by referring to the concentration in the pure buffer at the same pH. For example, at a pH of 4 there was a concentration of 0.0197 in the buffer but of 0.0464 in the sulfanilic acid. To get the contribution of the sulfanilic acid itself, the measured conductivity of the buffer is subtracted from that of the sulfanilic acid and buffer at the same pH. This resulting conductivity is partly due to the sulfanilic acid, and partly due to the excess of sodium ion.

TABLE I.

Buffer		Sulfanilic acid plus buffer		Lysin plus buffer		Mixture plus buffer	
pH	*k*	*pH*	*k*	*pH*	*k*	*pH*	*k*
3.77	590	3.75	1105	3.75	543	3.90	1078
4.04	591	4.50	1134	4.24	564	4.34	1104
4.37	590	5.72	1200	4.87	592	4.74	1130
6.28	718	5.85	1208	5.26	630	5.30	1165
6.78	833	6.33	1251	5.65	709	5.75	1221
7.27	936	6.63	1306	5.88	762	5.91	1220
7.77	995	6.88	1355	6.36	831	6.00	1214
8.70	1010	7.12	1400	6.83	932	6.21	1230
9.75	1026	7.57	1457	7.40	1002	6.41	1287
10.27	1049	7.93	1487	7.95	1044	6.61	1345
10.70	1090	8.68	1510	8.43	1065	6.83	1383
11.07	1175	10.20	1550	8.83	1080	7.03	1405
		10.40	1565	9.23	1110	7.27	1434
		11.07	1677	9.72	1162	7.83	1496
				10.42	1270	8.36	1539
				11.00	1425	8.62	1555
						9.42	1622
						10.10	1717
						11.12	1965

TABLE II.

pH	Buffer		Sulfanilic acid			Lysin			Mixture		
	Na	*k*	*Na*	*k*	*k'*	*Na*	*k*	*k'*	*Na*	*k*	*k'*
4.0	.0197	590	.0464	1117	527	.0137	554	−36	.0403	1085	495
5.0	.0207	613	.0484	1166	553	.0153	601	−12	.0428	1147	534
5.2	.0209	623	.0485	1175	552	.0158	624	1	.0431	1164	541
5.4	.0215	635	.0485	1180	545	.0168	645	10	.0438	1170	535
5.6	.0220	649	.0486	1190	541	.0176	694	45	.04415	1196	547
5.8	.0231	665	.0496	1205	540	.0192	756	91	.0452	1226	561
6.0	.0243	684	.0506	1224	540	.02115	771	87	.0465	1214	530
6.2	.0258	706	.05144	1236	530	.0232	800	94	.0479	1228	522
6.4	.0276	739	.0525	1262	523	.0259	838	99	.0498	1286	547
6.6	.0297	785	.0537	1300	515	.0290	890	105	.0518	1344	559
6.8	.0320	835	.05525	1338	503	.0312	929	94	.0537	1382	547
7.0	.0343	880	.0570	1375	495	.0336	955	75	.0557	1402	522
8.0	.0393	1002	.0612	1489	487	.0389	1046	44	.0605	1511	509
9.0	.040	1013	.0625	1514	501	.0429	1090	77	.0645	1578	565
10.0	.0405	1035	.0632	1538	503	.0472	1199	164	.0695	1701	666
11.0	.0436	1165	.0660	1666	501	.0556	1425	260	.078	1933	768

The values of the conductivities must be multiplied by 10^{-6} to give reciprocal ohms.

The difference in total sodium concentration can be at once obtained, but some assumption must be made as to the relation of the total sodium concentration to that of the sodium ion. Due to the necessity of this correction, the values of these conductivities are not presented as significant data in themselves, in fact their precise magnitude as well as their real significance is a question. However, the general shape of the curve obtained by plotting them against the pH is considered significant, and slight errors in the sodium ion correction will alter neither the general shape of the curve nor the position of the

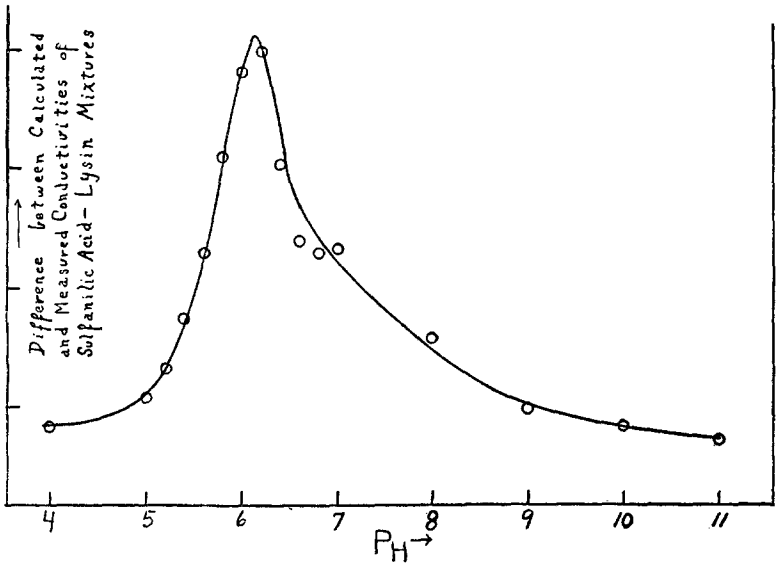

FIG. 1.

maximum in Fig. 1. Assuming, then, the isohydric principle, and the value 0.92 for the degree of ionization of the sodium salts, the conductivity correction for the sodium ion can be calculated from its ion conductance at 25°C., 50.7, and the cell constant. Table II, then, gives the total sodium concentration, the measured total conductivity in the column headed k, and the apparent conductivity of the substances studied, uncorrected for sodium ion concentration difference, in the column headed k'. The latter value is merely the difference between the conductivity of the solution and that of pure buffer at the same pH.

Table III gives the final corrected values for the contributions of sulfanilic acid, lysin, and their mixture to the total conductivity. The last column contains the differences obtained by subtracting the measured contribution of the mixture from the sum of the contributions of the two components when studied separately. These differences are plotted against pH in Fig. 1.

This curve, representing the pH function of the decrease in conductivity from what might be expected, due presumably to mutual binding of sulfanilic acid and lysin, passes through a fairly well defined

TABLE III.

pH	$k \times 10^6$				
	Sulfanilic acid	Lysin	Mixture (observed)	Mixture (calculated)	Difference
4.0	157	48	209	205	−4
5.0	167	63	227	230	3
5.2	169	72	231	241	10
5.4	170	75	225	245	20
5.6	172	106	239	278	39
5.8	172	145	254	317	63
6.0	175	131	222	306	84
6.2	174	130	215	304	89
6.4	177	123	239	300	61
6.6	179	115	252	294	42
6.8	180	105	246	285	39
7.0	180	85	225	265	40
8.0	183	50	215	233	18
9.0	188	37	225	225	0
10.0	188	71	263	259	−4
11.0	190	93	290	283	−7

maximum somewhere between the pH values 6.1 and 6.2. Calculation, by the method described in the preceding paper (1), of the value of the isoelectric point of this system, gives, using 7×10^{-4} for the acid ionization constant of sulfanic acid and 7×10^{-8} for the basic ionization constant of lysin, a pH of 6.03. The agreement seems quite satisfactory, considering the method of obtaining the experimental data and the problematical value of the basic ionization constant of lysin.

DISCUSSION.

While the above experiments were made on a comparatively simple system in order that a somewhat more definite interpretation might be possible, the more interesting and perhaps more obvious applications of the results are in connection with the much more complicated, though in many respects similar, systems which go to make up biological tissues.

One of the striking apparent anomalies which the point of view developed in this series of papers tends to straighten out is brought out in Fig. 2. From water absorption and behavior toward dyes Robbins (2) has found for the complex system potato tuber an isoelectric point at a pH of about 6, depending somewhat on the buffer used for adjusting the pH (Curve *C*). That this value is not even approximately characteristic of the protein most commonly associated with potato, namely tuberin, is apparent from the work of Cohn, Gross, and Johnson (3), who found for this protein an isoelectric point at a pH of about 4. Their tuberin was obtained from acid precipitation of potato juice. It is significant to note that they describe the precipitation of protein from potato juice by alkali as well as by acid. The latter precipitation reached a maximum at a pH of about 8, but was not otherwise studied. The solubility curve for the protein material in potato juice as a function of pH is given by Curve *A* of Fig. 2. (Curve *B* gives the same for carrot juice indicating similar behavior.) Both are taken from the work of Cohn, Gross, and Johnson. The point of maximum solubility between the two minima corresponds roughly with Robbins' isoelectric point of the system potato tuber, *i.e.* with the point of minimum water imbibition (Curve *C*). The two points are not exactly the same, but Robbins was working with whole tissue, while Cohn, Gross, and Johnson were working with the extracted juice.

The comparatively large specific effect of the particular buffering material employed on the isoelectric point of a complex system may also be expected, due to selective "binding tendencies" between the specific buffer ions and one or another of the components of the original system. Thus Robbins (2) finds a difference of nearly half a pH unit between the isoelectric points of potato tuber tissue as determined

by using citrate or phosphate buffer and as determined by phthalate buffer. In Fig. 2, Curve *C*, Curve I was obtained using phosphate adjustments and Curve II using phthalate adjustments.

The concept of such a mixed system offers also a possible chemical mechanism for the taking on of foods of both a basic and an acidic

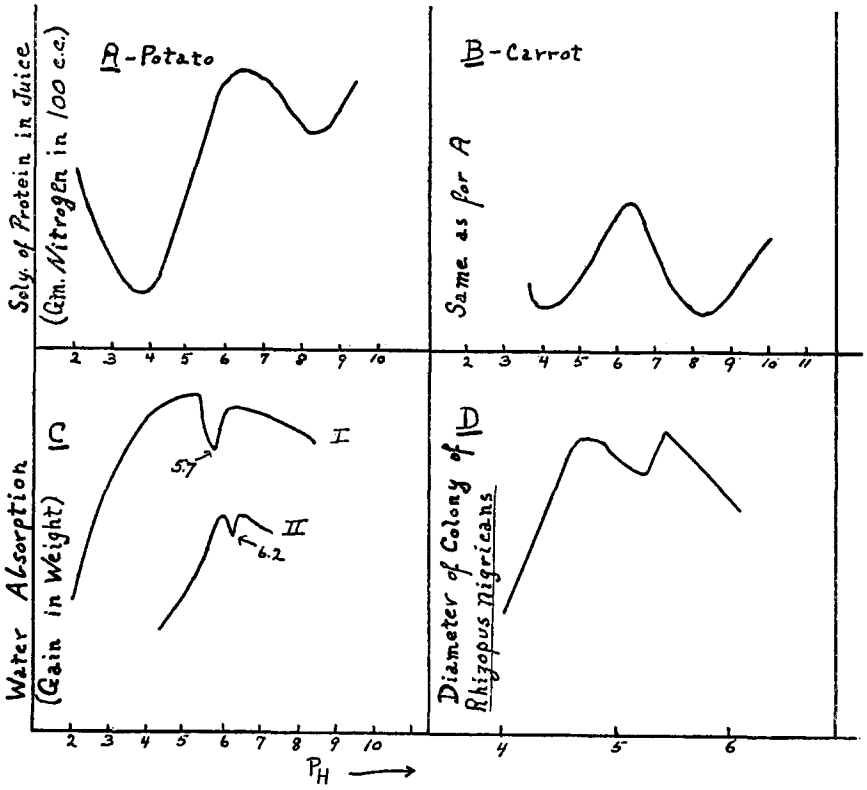

FIG. 2. Curve *A*, solubility of protein material in potato juice; Curve *B*, solubility of protein material in carrot juice (3); Curve *C*, water absorption by potato tuber tissue (2); Curve *D*, growth curve of *Rhizopus nigricans* on potato dextrose agar (4).

nature. Thus, in the case of a complex system, there will be a fair pH range through which one or the other of the components will exhibit a tendency to bind foods either of a carbohydrate or of a peptone nature. Growth curves over a fair pH range are suggestive on this point

(Fig. 2, Curve *D*) (4). At quite low or quite high pH values, nutrition, according to these curves, is very inefficient. Starting in acid solution, as the pH increases the rate of growth at first steadily increases. If the taking on of foods is primarily influenced by the ionic condition of the organism rather than by the ionic condition of the foods, and if the organism were acting as a simple ampholyte, we might expect an optimum condition for growth at its isoelectric point. (This would not be analogous to water imbibition.) At such a pH, in case of a simple ampholyte, the active anion concentration would be equal to the active cation concentration. In a mixed system, however, such is not the case when the lower isoelectric point, *i.e.* of one of the components, is reached, and actually the growth curve continues to rise, probably until the extent of mutual binding of the components of the system itself begins to affect results. The curve thus passes through a maximum and then descends to a minimum, probably at or near the point of maximum binding, *i.e.* the isoelectric point of the system. The rate of growth, even at this minimum, is higher than it is at those points corresponding more probably to the isoelectric points of the components, and the fact that it is a distinct minimum does not at all mean that growth is poor. From this minimum point, as one proceeds to higher pH values, the curve again rises, passing through another maximum, and then rapidly falls.

Work is now in progress to determine, if possible, the pH growth curves of organisms utilizing foods which might be considered entirely acidic in character, as well as foods which are entirely basic. Experiments on the specific effect of individual buffers are also under way, and it is hoped that soon the point of view developed here can be somewhat quantitatively tested out on systems somewhat more complicated than those herein described, but which are still sufficiently definitely known to permit of quantitative study and interpretation.

SUMMARY.

Conductivities of sulfanilic acid, lysin, and mixtures of the two were made over a wide pH range, the pH being adjusted by means of phosphate buffers. The actual conductivities of the sulfanilic acid, the lysin, and the mixture were calculated. The difference between the conductivity of the mixture and the sum of the conductivities of

the components alone passes through a maximum at a pH theoretically calculable as the isoelectric point of the system.

Certain applications of the results are made to the explanation of the behavior of living tissues.

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